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(71) Applicant (for all designated States except US): **ITAL-FARMACO S.P.A.** [IT/IT]; Viale Fulvio Testi, 330, I-20126 Milano (IT).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **MASCAGNI, Paolo** [IT/IT]; Via Dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **LEONI, Flavio** [IT/IT]; Via Dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **PORRO, Giulia** [IT/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **PAGANI, Paolo** [IT/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **DONA', Giancarlo** [IT/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **POZZI, Pietro** [IT/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **DINARELLO, Charles** [US/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **FANTUZZI, Giamila** [IT/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **SIEGMUND, Britta** [DE/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo

(IT). **REZNIKOV, Leonid** [US/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **BUFLER, Philip** [DE/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT). **KIM, Soo-Hyun** [KR/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT).

(74) Agents: **BANFI, Paolo** et al.; Bianchetti Bracco Minoja S.r.l., Via Rossini, 8, I-20122 Milano (IT).

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(54) Title: HISTONE DEACETYLASE ENZYME-INHIBITING DERIVATIVES OF HYDROXAMIC ACID AS NEW CYTOKINE SYNTHESIS-INHIBITING ANTI-INFLAMMATORY DRUGS

(57) Abstract: The use of derivatives of hydroxamic acid having histone deacetylase enzyme-inhibiting activity for the preparation of anti-inflammatory medicaments is disclosed.



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**HISTONE DEACETYLASE ENZYME-INHIBITING DERIVATIVES OF
HYDROXAMIC ACID AS NEW CYTOKINE SYNTHESIS-INHIBITING
ANTI-INFLAMMATORY DRUGS**

This invention relates to the use of hydroxamic acid derivatives having histone deacetylase enzyme-inhibiting activity for the preparation of anti-inflammatory medicaments.

Some derivatives of hydroxamic acid which inhibit histone
5 deacetylases are known. Those which have been most extensively studied are suberoylanilide hydroxamic acid (SAHA), N-hydroxy-3-[3-(hydroxyamino)-3-oxo-1-propenyl]-benzamide (CBHA) and trichostatin (TSA). Other derivatives are described in Proc. Natl. Acad. Sci. USA 95, 3003-3007, 1998; Tumori, 2001 Nov-Dec, 87 (6): S12-4; Anticancer Drugs, 2002 Jan, 13 (1): 1-
10 13; Nature Rev Cancer, 2001 Dec, 1 (3): 194-202; Curr Opin Oncol, 2001 Nov. 13 (6): 477-83; Cancer Chemother Pharmacol, 2001, Aug, 48 Suppl 1:S20-6; Cancer Chemother Pharmacol, 2001 Aug, 48 Suppl 1:S17-9; Haematologica, 2001 Sep; 86 (9): 908-17.

These compounds have mainly been studied as potential anti-tumoral
15 drugs: trichostatin, an antifungal antibiotic isolated from *Streptomyces hygroscopicus*, is a potent inducer of murine erythroleukaemic cell differentiation (Cancer Res. 47, 3288-3691, 1987), while SAHA and CBHA have been studied by the Sloan Kettering Institute (WO 95/31977) as tumour cell differentiation inducing agents.

20 The therapeutic use of histone deacetylase inhibitors to treat tumours is described in Anticancer Res. 20, 1471-1486, 2000 and Exp.Opin.Invest. Drugs 8(10),1611-1621,1999.

It has now been found that the known derivatives of hydroxamic acid having histone deacetylase inhibiting activity, especially trichostatin and

SAHA, inhibit the synthesis of pro-inflammatory cytokines, and can therefore be used to treat disorders which can be alleviated by inhibiting those cytokines. Examples of such disorders, with an inflammatory and/or autoimmune basis, include multiple sclerosis, Crohn's disease and ulcerative colitis, atherosclerosis, rheumatoid arthritis, psoriasis, spondyloarthropathies (an anchilosing spondilitis, psoriatic arthritis, arthritis connected to ulcerative colitis), AIDS-related neuropathies, asthma, chronic obstructive lung diseases, bronchitis, pleuritis, acute and chronic hepatitis (either viral, bacterial or toxic), acute glomerulonephritis and, broadly speaking, all disorders with an inflammatory component

For the therapeutic uses considered, the hydroxamic acid derivatives will be administered at doses ranging between 1 and 500 mg one or more times a day, depending on the disorder concerned and the pharmacotoxicological characteristics of the compound in question, which can be administered in the form of suitable oral, parenteral or topical formulations.

The following examples illustrate the invention in greater detail.

EXAMPLE 1- Inhibition of cytokine production *in vitro*

The treatment of lymphocytes with lipopolysaccharide (LPS) induces the production of various pro-inflammatory cytokines, such as TNF α , IL-1 β and IFN γ (J. Biol. Chem. 1990; 265(18): 10232-10237; Science, 1998; 281:1001-1005).

The effect of SAHA and TSA has been studied by evaluating the inhibitory effect of the compound on cytokine production by peripheral blood mononuclear cells (PBMC) from healthy volunteers (2 to 6 donors), stimulated with LPS.

Samples of peripheral blood or buffy coats from healthy volunteers were used. The samples were separated by centrifugation on density gradient

using Ficoll-Hypaque, and the PBMCs thus obtained were seeded in 96-well dishes (500,000 cells/well), incubated for 60 minutes with SAHA or TSA at various doses, and then stimulated with LPS from *E. coli* O55:B5 (10 ng/ml) for 24 hours in the presence of the compound. At the end of the treatment the
5 pro-inflammatory cytokines $\text{TNF}\alpha$, $\text{IL-1}\beta$ were measured by means of an electrochemiluminescence assay (ECL) using specific commercial antibodies.

Interferon γ ($\text{IFN}\gamma$) was measured with a commercially available ELISA assay.

Cytokine $\text{IFN}\gamma$ is produced by the T lymphocytes following their
10 stimulation by pro-inflammatory cytokines, especially IL-12 and IL-18 (Dinarello C. A. and Moldawer L. L. Proinflammatory and anti-inflammatory cytokines in rheumatoid arthritis. A primer for clinicians. 2nd Edition, Amgen Inc., 2000).

The effect of SAHA and TSA on $\text{IFN}\gamma$ synthesis induced by stimulating
15 PBMCs with IL-12 and IL-18 *in vitro* was evaluated on this basis. PBMCs were seeded in round-bottomed 96-well dishes (500,000 cells/dish), and incubated with various doses of SAHA or TSA for 60 minutes. At the end, the cells were stimulated for 48 hours in the presence of the compound by simultaneous addition of recombinant IL-12 (10 ng/ml) and recombinant IL-
20 18 (20 ng/ml). The quantity of $\text{IFN}\gamma$ produced was determined with a commercial ELISA assay.

The effect of the various doses of SAHA and TSA was measured as the percentage inhibition of production of the cytokine in question compared with untreated control cells. The concentration able to induce 50% inhibition of
25 cell growth (IC_{50}) was determined by linear regression.

The results are summarised in the table below:

Cytokine	SAHA IC ₅₀ (nM)	TSA IC ₅₀ (nM)
TNF α	200	50
IL-1 β	100	100
IFN γ	50	10
IFN γ (from IL-12 + IL-18)	740	490

These results clearly indicate that SAHA and TSA inhibit synthesis of all the inflammatory cytokines induced by LPS with an IC₅₀ in the nanomolar range (50-200nM).

- 5 SAHA and TSA also inhibit the synthesis of IFN γ by the T lymphocyte cells, as demonstrated by their efficacy (IC₅₀ 740 nM and 490 nM respectively) when the stimulus used was the combination of IL-12 and IL-18 specific for that cell line.

EXAMPLE 2 - Inhibition of cytokine production *in vivo*

- 10 Systemic administration of LPS to laboratory animals is known to induce rapid, massive production of pro-inflammatory cytokines (Immunopharmacol. 1992; 14(6): 1045-1050).

- Female BALB/c mice (20-22 grams) were treated orally with SAHA at the various doses indicated, then treated after 60 minutes with LPS from *E. Coli* O55:B5 (30 mg/Kg intraperitoneally). 90 minutes after the endotoxin administration, blood samples were taken from all the treated animals (10 animals/group), and the cytokines were measured with commercial ELISA assays.

- The results are set out in the table below, and expressed as the percentage inhibition of production of the cytokine in question:

20

TREATMENT	% inhibition of TNF α	Inhibition of IL-1 β	Inhibition of IL-6
SAHA			
0.1 mg/Kg	40	13	10
1 mg/Kg	53	15	3
10 mg/Kg	67	35	7
25 mg/Kg	68	37	25
50 mg/Kg	not done	51	29

The above results indicate that SAHA is active orally and able to inhibit, to a dose-dependent extent, pro-inflammatory cytokine synthesis induced *in vivo* in the mouse by administering endotoxin, thus confirming the results obtained *in vitro*.

EXAMPLE 3. Con A-Induced Liver Injury.

C57Bl6 mice were injected i.p. with either water vehicle or SAHA and after 1 h were injected i.v. with Con A as described in Proc. Natl. Acad. Sci. USA, (2000), 97, 2367-2372. After 24 h, serum amino-alanine transferase was measured.

Intravenous injection of Con A results in hepatic cell death within 12-24 h with markedly elevated serum levels of hepatic enzymes such as alanine amino transaminase (ALT). In mice pretreated with a single dose of SAHA (50 mg/kg) given i.p. 1 h before Con A, the 24-h level of serum ALT (mean \pm SE) was 8.144 ± 2.091 units/liter compared with 15.190 ± 2.580 in vehicle-treated mice ($n=6$ per group).

EXAMPLE 4. Nitric Oxide Production from Mouse Peritoneal Macrophages.

C57BLy6 mice were injected i.p. with 1 ml of sterile thioglycolate broth and killed after 5 days, and macrophages were isolated using instillation of 10 ml of ice-cold PBS into the peritoneal cavity. The cells were centrifuged (350 x g) and 3 ml of erythrocyte lysing reagent (PharMingen) was added for 10 min.

Seven milliliters of DMEM containing 5% FCS was added and the cells were centrifuged at 4°C. The cells were resuspended in DMEM at 1 million per ml and 0.5 ml were added to wells of a 48-well plate. SAHA was added for 60 min at 37°C and then stimulated with the combination of TNF- α plus IFN- γ . After 24 h, NO levels in the supernatant were determined using the Griess reagent as described in Am.J.Physiol.Cell Physiol. (2001), 280, C441-C450.

As shown in Fig. 1, SAHA inhibited NO production; at 200 nM, there was a 50% reduction (P , 0.05). Further reductions of 80 and 85% were observed at 400 and 800 nM, respectively.

EXAMPLE 5. Inhibition of IL-12 production by cultured monocytes.

Venous blood was obtained from consenting adults and separated over Ficoll-Hypaque. The PBMC fraction was washed and adjusted to five million cells per ml. Five hundred microliters was aliquoted into each well of 24-well flat-bottom plates, 100 μ l of SAHA was added, and the plates were incubated for 1 h at 37°C. The cells were stimulated with LPS, soluble OKT3, or cytokines, and after 24 or 48 h At 37°C the supernatant was removed and frozen for cytokine assays. Monocytes were isolated by centrifugation over Percoll, washed, suspended in RPMI with 10% FCS, and aliquoted at 2 million cells per ml in Petriperm Teflon-coated culture dishes (Sigma). The ELISA for human IL-12 (p70) was purchased from Endogen (Woburn, MA).

As shown in Fig. 2, there was a dose-dependent reduction in LPS/IFN- γ -induced IL-12 production in nonadherent human monocytes. At 200 nM, the reduction was 55% (P , 0.01) and at 86% at 400 nM (P , 0.001).

EXAMPLE 6 Dextran-induced colitis

Female, 8 week-old C57BL/6 mice (The Jackson Laboratories, Bar Harbor, ME) weighing 20-22 g were used in this study. The animals were housed in rooms at a controlled temperature and a 12 h day/night rhythm.

They were fed standard mice chow pellets ad libitum, had free access to tap water supplied in bottles, and were acclimatized to the conditions at least seven days before they were used in experiments. Mice were killed by cervical dislocation under isoflurane anesthesia (Fort Dodge, Iowa City, IA).

5 Mice were fed 3.5% dextran sulfate sodium (DSS; molecular weight 40 kDa; ICN, Aurora, OH) dissolved in sterile, distilled water ad libitum from day one to five followed by a five day observation period. SAHA was administered once daily orally (p. o.) in a total volume of 200 μ l and a concentration of 10 mg/kg body weight (BW) throughout the experiment (day 10 1 to 10). Control mice had free access to water and received either SAHA (10 mg/kg BW) or water p. o. once daily for 10 days.

Body weights, occult blood or the presence of gross blood per rectum, and stool consistency were determined daily. Two investigators blinded to the protocol assessed the clinical score (table 1). Weight loss < 1% compared to 15 day 1 was counted as 0 points, weight loss of 1 to < 5% as 1 point, 5 to < 9.9% as 2 points, 10 to 20% as 3 points and more than 20% as 4 points. For stool consistency, 0 points were given for well-formed pellets (formed), 2 points for pasty and semi-formed stools which did not stick to the anus (soft), and 4 points for liquid stools that did stick to the anus (diarrhea). Bleeding 20 was scored 0 points for no blood in hemoccult, 2 points for positive hemoccult, and 4 points for gross bleeding. These scores (body weight, stool consistency, rectal bleeding) were added and divided by 3 resulting in a total clinical score ranging from 0 (healthy) to 4 (maximal activity of colitis).

25 *Post mortem* (on day 10), the entire colon was removed from the caecum to the anus and the colon length was measured as an indirect marker of inflammation. Colon length has been shown to be a reliable parameter in this model as DSS-induced colitis is associated with colon shortening as described previously [Gastroenterology, 1990, 98, :694; J. Pharmacol. Exp. Ther.,

2001, 296:99-105].

From the results, reported in the following Tables 1-6, it is evident that SAHA effectively counteracts dextran-induced colitis, a valid and established model of inflammatory bowel diseases in humans.

5 Table 1. Clinical activity score [Lab. Invest., 1993, 69:238-249].

Score points	Weight loss	Stool consistency	Rectal bleeding
0	0%	Formed	Negative hemoccult
1	(>0%) <5%		
2	5-9.9%	Soft	Positive hemoccult
3	10-20%		
4	>20%	Diarrhea	Macroscopic bleeding

Table 2. Weight

Group	Days									
	1	2	3	4	5	6	7	8	9	10
DSS + SAHA	19.1±0.2	18.8±0.2	19.1±0.3	18.4±0.4	18.4±0.4	18.7±0.3	18.0±0.4	16.9±0.3	16.6±0.4	16.6±0.4
DSS + water	18.7±0.5	19.0±0.5	19.4±0.5	18.9±0.5	18.9±0.5	18.8±0.6	18.8±0.6	16.6±0.5	16.8±0.5	16.8±0.5
SAHA	18.9±0.3	18.8±0.4	18.9±0.2	18.9±0.3	19.0±0.1	19.0±0.3	18.9±0.1	19.0±0.3	19.1±0.1	19.1±0.2
Water	19.0±0.2	19.0±0.3	18.9±0.1	19.0±0.2	19.0±0.4	19.0±0.2	19.1±0.2	19.1±0.1	19.1±0.3	19.1±0.1

Table 3. Stool consistency as score

Group	Days									
	1	2	3	4	5	6	7	8	9	10
DSS + water	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.4	1.2 ± 0.4	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	1.6 ± 0.4	1.6 ± 0.4
DSS + SAHA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.4	0.8 ± 0.4	2.0 ± 0.0	2.0 ± 0.0	1.2 ± 0.4	1.2 ± 0.4
SAHA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Water	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 4. Bleeding

Group	Days									
	1	2	3	4	5	6	7	8	9	10
DSS + water	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.4	1.6 ± 0.4	2.8 ± 0.4	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	1.6 ± 0.4
DSS + SAHA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.2 ± 0.4	2.4 ± 0.4	2.0 ± 0.0	1.6 ± 0.4	1.2 ± 0.4	0.8 ± 0.4
SAHA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Water	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 5. Complete clinical score

Group	Days									
	1	2	3	4	5	6	7	8	9	10
DSS + water	0.0 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.8 ± 0.1	1.1 ± 0.2	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	1.9 ± 0.2
DSS + SAHA	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.7 ± 0.3	1.3 ± 0.3	1.5 ± 0.1	1.9 ± 0.1	1.5 ± 0.4	1.3 ± 0.4
SAHA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0
Water	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

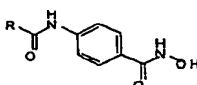
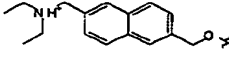
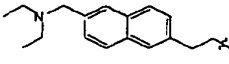
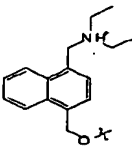
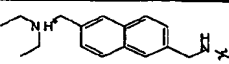
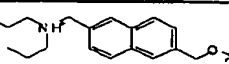
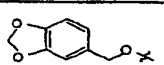
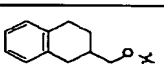
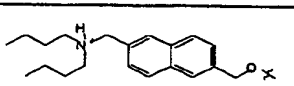
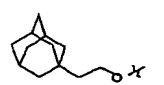
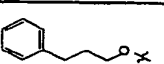
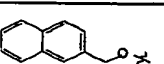
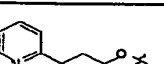
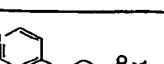

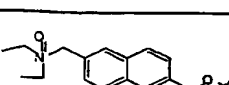
Table 6. Colon length

Group	Colon length (cm)
DSS + water	7.6 ± 0.2
DSS + SAHA	9.2 ± 0.2
SAHA	10.1 ± 0.2
Water	10.5 ± 0.3

EXAMPLE 7

5 A series of hydroxamic acid derivatives described in EP 901465 were subjected to the histone deacetylase (HDAC) and TNF α inhibition tests in accordance with the methods described by Lechner et al., Biochim Biophys. Acta 1996, 1296, 181-188 and Moreira A.L. et al., J. Exp. Med. 1993, 177, 1657-1680 respectively.

10 The results, set out in the following table, show that a linear correlation exists between the ability of these compounds to inhibit the synthesis of TNF α and their inhibition of HDAC activity.

	 General structure R	HDAC		TNF	
		IC50 nM	Potency	IC50 nM	Potency
1		20,0	100,00	7,0	100,00
2		62,0	32,26	10,2	68,75
3		65,0	30,77	10,3	68,02
4		78,0	25,64	11,2	62,66
5		46,7	42,86	12,7	55,28
6		80,0	25,00	50,0	14,00
7		91,0	21,98	65,5	10,68
8		133,3	15,00	67,8	10,32
9		600,0	3,33	159,1	4,40
10		105,0	19,05	159,1	4,40
11		8,1	246,91	159,1	4,40
12		260,0	7,69	230,0	3,04
13		260,0	7,69	270,0	2,59
14		86,7	23,08	300,0	2,33
15		206,7	9,68	1000,0	0,70

CLAIMS

1. The use of hydroxamic acid derivatives having histone deacetylase inhibiting activity for the preparation of anti-inflammatory medicaments.
- 5 2. Use as claimed in claim 1, wherein the derivatives are selected from suberoylanilide hydroxamic acid (SAHA), N-hydroxy-3-[3-(hydroxyamino)-3-oxo-1-propenyl]-benzamide (CBHA) and trichostatin (TSA).
3. Use as claimed in claim 2, wherein the derivatives are selected from suberoylanilide hydroxamic acid (SAHA) and trichostatin (TSA).
- 10 4. Use as claimed in claim 1 or 2 for the preparation of medicaments for the treatment of multiple sclerosis, Crohn's disease and ulcerative colitis, atherosclerosis, rheumatoid arthritis, psoriasis, spondyloarthropathies (anchilosating spondilitis, psoriatic arthritis, arthritis connected to ulcerative colitis), AIDS-related neuropathies, asthma, chronic obstructive lung diseases,
15 bronchitis, pleuritis, acute and chronic hepatitis (either viral, bacterial or toxic), acute glomerulonephritis.

1/2

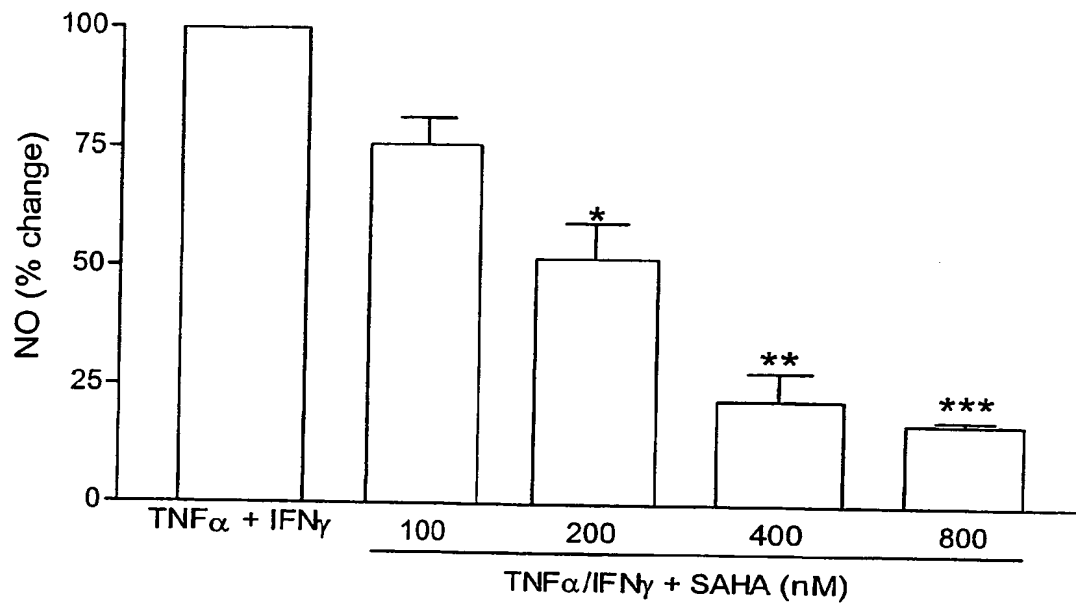


Fig. 1

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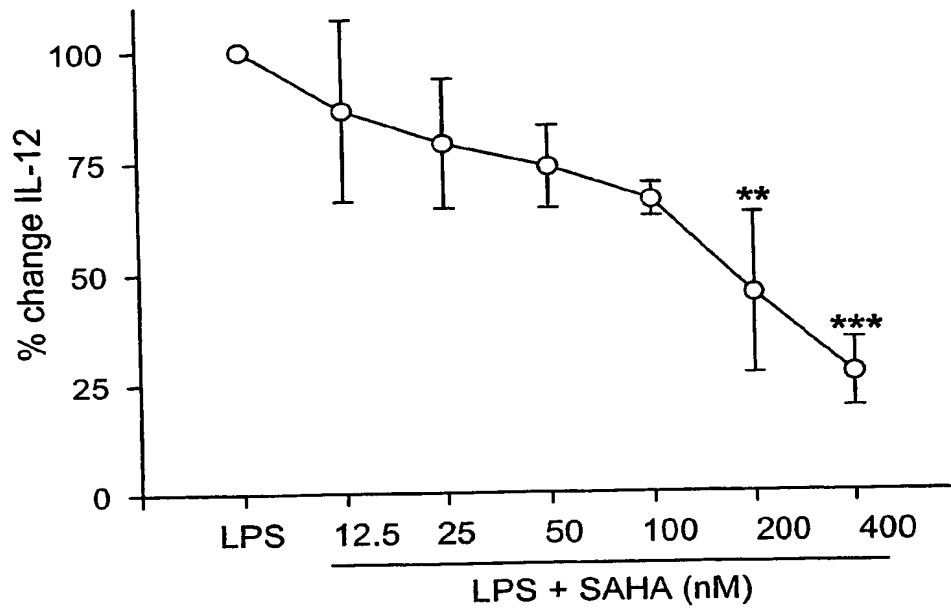


Fig. 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/08379

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/166 A61P19/02 A61P25/02 A61P29/00 A61P1/04
A61P9/10 A61P11/00 A61P11/06 A61K31/165

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, WPI Data, PAJ, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 35990 A (JAMISON TIMOTHY F ;HARVARD COLLEGE (US); TAUNTON JACK (US); HASSIG) 2 October 1997 (1997-10-02) page 11, line 5-8; figure 1A; example 1 page 82, line 14 -page 83, line 9	1-4
X	HUANG N ET AL: "INHIBITION OF IL-8 GENE EXPRESSION IN CACO-2 CELLS BY COMPOUNDS WHICH INDUCE HISTONE HYPERACETYLATION" CYTOKINE, ACADEMIC PRESS LTD, PHILADELPHIA, PA, US, vol. 9, no. 1, January 1997 (1997-01), pages 27-36, XP001013211 ISSN: 1043-4666 abstract page 28, column 1, line 14-17	1-4

-/--

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 6E1 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Ansaldo, M

INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MISHRA NILAMADHAB ET AL: "Histone deacetylase inhibitor Trichostatin A as a strong candidate for treatment of systemic lupus erythematosus." FASEB JOURNAL, vol. 15, no. 5, 8 March 2001 (2001-03-08), page A1214 XP009002039 Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001;Orlando, Florida, USA; March 31-April 04, 2001 ISSN: 0892-6638	1-4
Y	abstract	1-4
Y	----- RICHON VICTORIA M ET AL: "A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 6, 17 March 1998 (1998-03-17), pages 3003-3007, XP001120542 March 17, 1998 ISSN: 0027-8424 abstract; table 1	1-4
P,X	----- WO 02 055017 A (MISHRA NILAMADHAB ;KAMMER GARY M (US); UNIV WAKE FOREST (US)) 18 July 2002 (2002-07-18) page 6, line 7-11; claims 1,3,5,6,10-16 page 6, column EMB-2002, line 20 -page 7, line 17	1-4
X	----- EP 0 574 758 B (HOFFMANN LA ROCHE) 22 December 1993 (1993-12-22) page 2, line 41-43; claims 1,23	1,4
X	----- EP 0 757 984 B (ONO PHARMACEUTICAL CO) 12 February 1997 (1997-02-12) claim 7	1,4
X	----- WO 97 43251 A (BERTOLINI GIORGIO ;PAVICH GIANFRANCO (IT); BIFFI MAURO (IT); ITALF) 20 November 1997 (1997-11-20) page 2, line 3 - line 7; claims 1,4,6	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP 02/08379

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9735990	A	02-10-1997	AU 2990597 A WO 9735990 A2	17-10-1997 02-10-1997
WO 02055017	A	18-07-2002	WO 02055017 A2	18-07-2002
EP 0574758	B	22-12-1993	AT 170840 T AU 659555 B2 AU 3981693 A BG 61724 B1 BG 97857 A CA 2098168 A1 CN 1083062 A ,B CZ 9301081 A3 DE 69320869 D1 DE 69320869 T2 DK 574758 T3 EP 0574758 A1 ES 2121896 T3 FI 932692 A HR 930978 A1 IL 105921 A JP 2039885 C JP 6065196 A JP 7076210 B MX 9303391 A1 NO 932117 A NZ 247765 A PH 30245 A PL 299261 A1 RO 112613 B SI 9300289 A SK 57393 A3 US 5318964 A US 5447929 A ZA 9303957 A	15-09-1998 18-05-1995 16-12-1993 30-04-1998 15-11-1994 12-12-1993 02-03-1994 16-02-1994 15-10-1998 29-04-1999 07-06-1999 22-12-1993 16-12-1998 12-12-1993 30-04-1997 04-01-1998 28-03-1996 08-03-1994 16-08-1995 30-06-1994 13-12-1993 27-11-1995 05-02-1997 10-01-1994 28-11-1997 31-12-1993 11-05-1994 07-06-1994 05-09-1995 13-12-1993
EP 0757984	B	12-02-1997	AT 226936 T DE 69624536 D1 EP 0757984 A1 JP 9104672 A KR 231230 B1 US 6022893 A	15-11-2002 05-12-2002 12-02-1997 22-04-1997 15-11-1999 08-02-2000
WO 9743251	A	20-11-1997	IT MI960968 A1 AU 713300 B2 AU 2896497 A BR 9709234 A CA 2254066 A1 CN 1221403 A CZ 9803667 A3 DE 69703207 D1 DE 69703207 T2 DK 901465 T3 WO 9743251 A1 EP 0901465 A1 ES 2151267 T3 GR 3035128 T3 JP 2000510472 T	14-11-1997 25-11-1999 05-12-1997 10-08-1999 20-11-1997 30-06-1999 16-06-1999 02-11-2000 01-02-2001 18-12-2000 20-11-1997 17-03-1999 16-12-2000 30-04-2001 15-08-2000

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International

Application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9743251	A	KR 2000010982 A	25-02-2000
		PL 329873 A1	12-04-1999
		PT 901465 T	31-01-2001
		RU 2177473 C2	27-12-2001
		SK 157998 A3	13-04-1999
		US 6034096 A	07-03-2000

Form PCT/ISA/210 (patent family annex) (July 1992)

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31/165

54, I-20092 Cinisello Balsamo (IT). KIM, Soo-Hyun
[KR/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Bal-
samo (IT).

(21) International Application Number:
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(74) Agents: PISTOLESI, Roberto et al.; Dragotti & Asso-
ciati Srl, Galleria San Babila 4/C, I-20122 Milano (IT).

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(71) Applicant (*for all designated States except US*): ITAL-
FARMACO S.P.A. [IT/IT]; Viale Fulvio Testi, 330,
I-20126 Milano (IT).

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(72) Inventors; and

(75) Inventors/Applicants (*for US only*): MASCAGNI, Paolo
[IT/IT]; Via Dei Lavoratori, 54, I-20092 Cinisello Balsamo
(IT). LEONI, Flavio [IT/IT]; Via Dei Lavoratori, 54,
I-20092 Cinisello Balsamo (IT). PORRO, Giulia [IT/IT];
Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT).
PAGANI, Paolo [IT/IT]; Via dei Lavoratori, 54, I-20092
Cinisello Balsamo (IT). DONA', Giancarlo [IT/IT];
Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT).
POZZI, Pietro [IT/IT]; Via dei Lavoratori, 54, I-20092
Cinisello Balsamo (IT). DINARELLO, Charles [US/IT];
Via dei Lavoratori, 54, I-20092 Cinisello Balsamo (IT).
POMERANTZ, Benjamin [US/IT]; Via dei Lavoratori, 54,
I-20092 Cinisello Balsamo (IT). FANTUZZI, Giamila
[IT/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Balsamo
(IT). SIEGMUND, Britta [DE/IT]; Via dei Lavoratori,
54, I-20092 Cinisello Balsamo (IT). REZNIKOV, Leonid
[US/IT]; Via dei Lavoratori, 54, I-20092 Cinisello Bal-
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(54) Title: HISTONE DEACETYLASE ENZYME-INHIBITING DERIVATIVES OF HYDROXAMIC ACID AS NEW
CYTOKINE SYNTHESIS-INHIBITING ANTI-INFLAMMATORY DRUGS

(57) Abstract: The use of derivatives of hydroxamic acid having histone deacetylase enzyme-inhibiting activity for the preparation
of anti-inflammatory medicaments is disclosed.